Liquid Chromatography–Electrochemical Detection of Furazolidone and Metabolite in Extracts of Incurred Tissues

One-day-old chicks were raised to maturity on a diet fortified with 0.0055% furazolidone. Analyses of tissue extracts by a liquid chromatographic-electrochemical detection screening procedure for nitro-containing drugs disclosed, in addition to the parent drug, an unidentified metabolite in the liver and breast tissue of the mature birds sacrificed while on the fortified feed. No evidence of residues of the drug or metabolite was found in birds removed from the medicated feed 48 h prior to sacrifice. In view of the rapid in vivo and postmortem metabolism of the parent drug in liver tissue, the metabolite can serve as an alternative means of detecting furazolidone residues in chicken tissues.

Furazolidone [*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone] is used as a growth promoter and/or antimicrobial agent in poultry feeds (1). The drug and/or its metabolites are known to produce tumors in laboratory animals and are suspected of being carcinogenic to humans (2). While zero tolerance levels had been established for total residues of furazolidone in animal tissues in the past, the Food and Drug Administration is presently taking steps to ban the drug completely for animal use (3). It is imperative, therefore, that sensitive methods to detect residues in tissue be available.

Several methods have been reported (4–7) to detect furazolidone at the low ppb level in poultry tissues. However, the parent drug is rapidly metabolized in vivo to a variety of metabolites (for the most part uncharacterized) that tend to persist in poultry tissues longer than the parent drug and are not detected by the previously published methods (8–10).

Recently, we reported (11) a multiresidue screening procedure for 6 nitro-containing drugs, including furazolidone, at the low ppb level in chicken tissues. Applying that procedure to tissues from birds maintained continuously on furazolidone-fortified feed prior to sacrifice, we detected a metabolite in extracts of liver and breast tissue. The present paper reports results of those studies as well as in vitro studies on liver tissues fortified with furazolidone.

METHOD

Reagents and Apparatus

(a) *Solvents.*—Same as Ref. 11.

(b) *Drugs.*—Furazolidone (Norwich-Eaton Pharmaceuticals, Norwich, NY 13815); Furox® 50 medicated premix containing 50 g furazolidone/lb (Smith Kline Animal Health Products, Division of Smith Kline Beckman Co., 1600 Paoli Pike, PO Box 2650, West Chester, PA 19380).

(c) *Tissue homogenizer.*—Same as Ref. 11.

(d) *Centrifuge.*—Same as Ref. 11.

(e) *Sand.*—Same as Ref. 11.

(f) *Neutral alumina.*—Same as Ref. 11.

(g) *Liquid chromatograph-electrochemical detector.*—Same as Ref. 11, except glassy carbon electrode at −0.8 V vs Ag/AgCl, 5–10 μA full scale.

Feeding Trials

One-day-old male broiler chicks were placed on a commercial-type starter-grower diet fortified with 0.0055% furazolidone. At 42 days of age, 9 birds were sacrificed. An additional 9 medicated birds were removed to a control feed for 2 days, then sacrificed. Immediately after sacrifice, livers were removed, placed in a plastic bag and frozen in liquid nitrogen to limit postmortem metabolism of the drugs. Breast and thigh muscle were removed as quickly as possible and frozen to less than −50°C. All tissues were maintained at <−50° to −20°C prior to analyses. Tissues removed from 9 birds raised for 42 days on a nonmedicated feed served as controls.

Tissue Preparation

Frozen tissue was partially thawed, cubed, and blended in a chilled Waring blender. The blended tissue was quickly packed in plastic bags and refrozen in dry ice. Two livers were blended together as a result of problems initially encountered in blending a single liver (Liver 828). Breast and thigh muscle were blended individually.

Quantitation

From stock solutions of furazolidone in dimethylformamide (DMF), prepare (daily) standard solutions containing approximately 2.0, 10.0, 20.0, and 40.0 ng/2 mL of pH 6.0 phosphate buffer–methanol (1 + 1). Determine average detector response factor (Rf) by relating concentration to measured peak heights (ng/mm). Concentration of furazolidone in incurred tissue is determined by the following formula:

$$\text{Concn (ppb)} = [\text{Rf (ng/mm)} \times \text{peak height (mm)}]/[1.875\ \text{g of tissue} \times 0.9]$$

where 0.9 represents percent recovery from fortified tissues (11). Duplicate samples were analyzed on different days.

In vitro Studies

Frozen, ground, control liver (2.5 g) was fortified with 2.5 μL of a 0.2 μg/μL DMF solution of furazolidone (200 ppb) in a 50 mL polyethylene centrifuge tube. The fortified tissue was warmed to 32 or 39°C in a water bath and held for

Table 1. Concentration of furazolidone in tissues of zero time birds

Bird	Concentration, ppb		
	Liver	Thigh	Breast
828	0.57 ± 0.23	ND[a]	2.89 ± 0.28
821		0.96 ± 0.21	ND
	0.76 ± 0.07[b]		
844		0.87 ± 0.00	0.72 ± 0.08
834		ND	ND
	0.53 ± 0.08[c]		
837		tr[d]	ND
853		1.21 ± 0.04	tr
	1.00 ± 0.21[e]		
859		ND	tr
863		ND	ND
	1.06 ± 0.01[f]		
871		1.48 ± 0.58	0.91 ± 0.0

[a] ND = not detected.
[b] Based on blending of samples 821 and 844.
[c] Based on blending of samples 834 and 837.
[d] tr = trace amounts.
[e] Based on blending of samples 853 and 859.
[f] Based on blending of samples 863 and 871.

varying lengths of time. Following incubation, the tissue was frozen in dry ice, held 18 h at −20°C, and then subjected to the extraction procedure.

Results and Discussion

Table 1 summarizes results of duplicate samples of tissues from birds sacrificed while on furazolidone-fortified feeds (zero time birds). With few exceptions, namely Liver 828 and Thigh 871, duplicate samples were generally in good agreement. No furazolidone was detected in birds removed from the medicated feed 2 days prior to sacrifice, and no interfering peaks at the retention time of furazolidone were observed in control tissues. The low concentration of the parent drug in zero time tissues was not unexpected because furazolidone is rapidly metabolized in vivo. Sugden et al. (7) reported furazolidone levels of 10 and 20 ppb in thigh muscle and zero concentration in livers 8 h following dosing of chickens with 50 mg furazolidone. The birds in this study (av. wt 2.1 kg) consumed a total of 8 mg furazolidone on the average (148 g feed) in the 24 h period prior to sacrifice. Although the need to combine livers for rapid and thorough blending limits a complete comparison of tissues of individual birds, results on breasts and thigh muscles suggest that differences between birds and tissues of the same bird do exist.

Figure 1 shows typical chromatograms of liver extracts from birds maintained on the nonmedicated control feed, birds fed furazolidone-fortified feed continuously until sacrificed, and birds fed furazolidone continuously but removed from the medicated feed 2 days prior to sacrifice. In addition to a peak at the retention time of furazolidone (4.9 min), chromatograms of liver extracts of zero time birds are characterized by the presence of a peak at 3.8 min. Because the peak is present only in liver extracts of zero time birds, it is concluded that the peak represents an unknown metabolite of furazolidone. The metabolite was also present, but to a much lesser extent, in several breast tissues of zero time birds; however, it was not detected in thigh muscle.

Table 2 shows the relative concentration of the unknown metabolite in liver tissues upon initial analyses. Because the

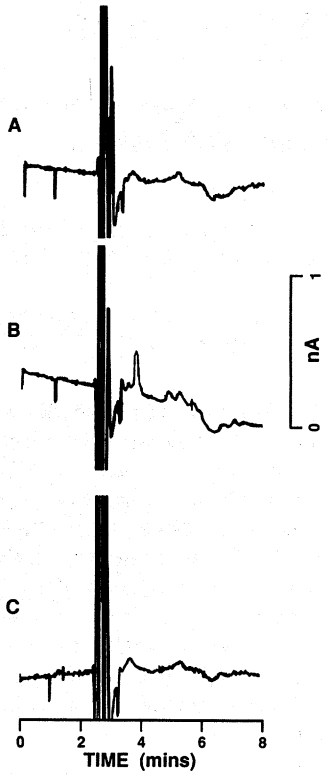

Figure 1. LC chromatograms of liver extracts from (A) birds maintained on nonmedicated control feed, (B) birds 853–859 fed furazolidone-fortified feed continuously until sacrificed, and (C) birds fed furazolidone-fortified feed continuously then moved to control feed 2 days prior to sacrifice. Electrochemical detection: potential −0.8 V; attenuation 5 nA full scale.

metabolite was unknown, concentrations were arbitrarily based on comparing the peak height with those of known concentrations of furazolidone and a 65% recovery as calculated from a comparison of peak heights from 3 successive extractions of single tissues. Subsequent studies on liver tis-

Table 2. Relative concentration of furazolidone metabolite in livers of zero time birds[a,b]

Liver	Relative concn, ppb
828	6.95
821	
	4.34[c]
844	
834	
	3.22[d]
837	
853	
	4.80[e]
859	
863	
	3.78[f]
871	

[a] Based on comparing peak heights of unidentified metabolite with peak heights of known concentrations of furazolidone.
[b] Corrected for 65% recovery.
[c] Based on blending of samples 821 and 844.
[d] Based on blending of samples 834 and 837.
[e] Based on blending of samples 853 and 859.
[f] Based on blending of samples 863 and 871.

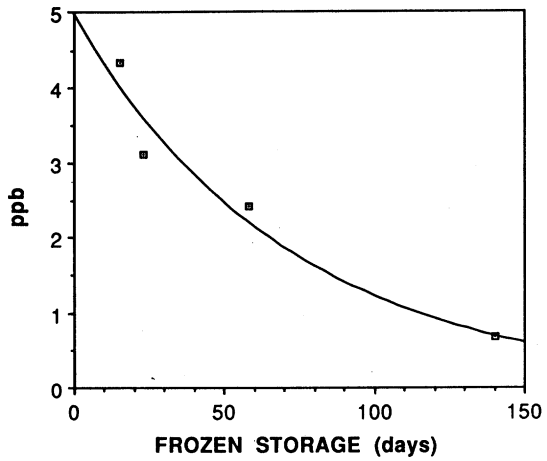

Figure 2. Exponential depletion of furazolidone metabolite in liver tissue samples 821–844 during frozen storage (~ = 0.990).

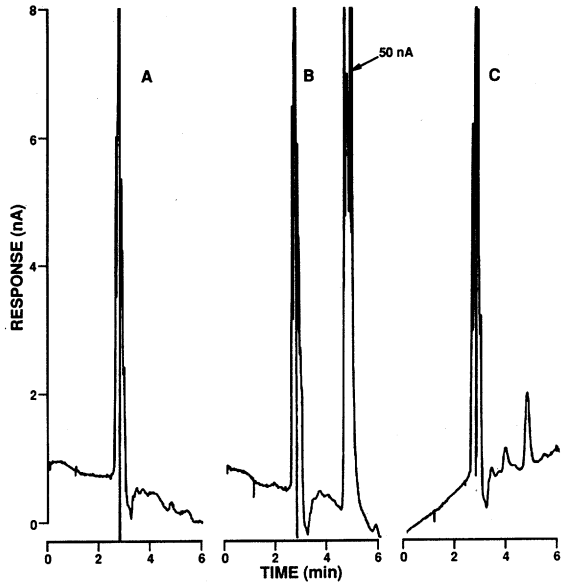

Figure 3. LC chromatograms of extracts of (A) frozen liver control; thawed incubated at 32°C for 15 min, and refrozen prior to extraction (attenuation 10 μA full scale), (B) furazolidone-fortified frozen liver; held at −20°C prior to extraction (attenuation 10 μA and 50 μA full scale), and (C) furazolidone-fortified frozen liver; thawed, incubated at 32°C for 15 min, and refrozen prior to extraction (attenuation 10 μA full scale), electrochemical detection; potential −0.8 V.

sues revealed that the metabolite depleted exponentially during frozen storage at −20°C (Figure 2). Because the liver tissues had been in frozen storage 15 to 17 days prior to initial analysis, the relative concentrations of the metabolite at the time of sacrifice were presumably higher than recorded. Breast 828, the tissue with the highest observed furazolidone concentration of all tissues analyzed, contained the only accurately measurable amount (1.88 ppb) of the metabolite among the positive breast tissues. It is not known whether the metabolite was present in measurable amounts in all breast tissues (as well as in thigh muscle of zero time birds) at the time of sacrifice but was depleted in frozen storage prior to initial analysis (25–40 days).

In contrast to depletion of the metabolite in frozen storage, in situ studies on incurred liver tissues showed that further metabolism of the unknown did not occur during incubation of the tissue at room temperature (23°C) for 1.5 h. Furazolidone itself is rapidly metabolized in situ, as well as in fortified liver tissues, with the appearance of a peak at the retention time of the unknown metabolite (Figure 3). However, whereas >95% of the furazolidone (200 ppb) was metabolized in vitro at 32°C for 15 min (>75% at 39°C for 5 min), the metabolite peak represented only 2% of the original peak height of the parent drug. Once again, further incubation at 32°C did not decrease concentration of the metabolite, suggesting that the metabolite was a minor (but relatively stable) end product of the metabolic process. Whether the metabolite observed in incurred tissues is the result of in vivo and/or postmortem metabolism in the short interval before freezing remains to be determined. It remains unidentified as well.

The results of this study show that the multiresidue screening procedure for nitro-containing drugs in chicken tissues can detect an unknown metabolite for furazolidone at the low ppb level. In view of the rapid in vivo and postmortem metabolism of the parent drug in liver tissue, the metabolite offers an alternative means for detecting furazolidone residues in chicken tissues.

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